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LARVICIDAL EFFICACY OF LEAF EXTRACTS OF *Rhanterium epapposum* Oliv. AGAINST DENGUE FEVER MOSQUITO VECTOR LARVAE, *Aedes aegypti* L., (DIPTERA: CULICIDAE)

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ABSTRACT

Dengue fever, transmitted by *Aedes aegypti* L. (Diptera: Culicidae), is the most important disease affecting human health and economy in the western part of Saudi Arabia. This study aimed to investigate the larvicidal activities of ethanolic, acetonic and aqueous extracts prepared from the leaves of Arfaj (*Rhanterium epapposum* Oliv.) against dengue fever mosquito vector, *Aedes aegypti*, under laboratory conditions. The larvicidal potency of ethanolic extract at high concentration of 50000 ppm was up to 98% after 24 h exposure. In contrast, acetonic and aqueous extracts caused 72 and 30% larval mortality, respectively, at the same concentration and exposure time. After 48 h exposure, the mortalities increased by 99, 86 and 35%, respectively, for ethanolic, acetonic and aqueous extracts. This study showed that the ethanolic extract had a higher larvicidal effect with lower LC_{50} value (168.15 ppm), followed by acetone (847.75 ppm) and aqueous (2278.22 ppm) extracts after 24 h exposure. Further studies are needed to isolate and identify the primary component responsible for larvicidal efficiency of this plant extract which may give good insights for developing a sustainable mosquito control method.

Keywords: Botanical pesticides, dengue fever vector, ethanolic extract, mosquito control, *Rhanterium epapposum*

INTRODUCTION

Worldwide, the transmission of several diseases to humans usually occurs by the propagation of mosquito-borne diseases while feeding a female mosquito on human blood. Malaria, dengue fever, West Nile fever, and lymphatic filariasis are some of the most dangerous diseases that some mosquito species can transmit. In addition, mosquitoes cause harassment to vocational, entertaining and public events. From more than 300 different species of mosquitoes that have been identified, only a few species are known to be medically important to human health. Universally, *Anopheles gambiae* Giles, *Culex pipiens* L. and *Aedes aegypti* L. are medically the most important species of mosquitoes (Wang *et al*., 2012; Karthika *et al*., 2018; Zheng, 2020). In tropical, subtropical, and temperate zones, dengue fever and dengue hemorrhagic fever, transmitted by mosquitos, is prevalent in more than 100 countries (Murray *et al.*, 2013; Yuan *et al*., 2020). These diseases cause public health risks to more than 2.5 billion people globally, with about 80 million people infected annually, accounting for 4% infection rate (Murray *et al.*, 2013; Yuan *et al*., 2020). In the western part of Saudi Arabia, especially in Jeddah province, the second-largest city and the main port of entry into the country, the significant impacts of dengue fever on human health and the economy are widely felt (Alshehri, 2013; Alwafi., 2013, Asiry *et al*., 2017).

Most mosquito control programs rely on applying insecticides to mosquitoes' breeding locations by targeting their immature stages (larval and pupal stages). However, the application of insecticides may sometimes be impractical and cause several environmental concerns like water pollution that negatively impacts human households and threaten human existence at provincial and municipal regions (Lacey, 1990; Sarker *et al.*, 2021). However, the effects of chemical insecticides on mosquito vector populations are usually transitory because vectors can rapidly develop resistance against them (WHO, 2009; Lui, 2015; Navridis *et al.*, 2018). Therefore, researchers have sought alternative methods to decrease the reliance on synthetic pesticides for mosquito control. Over past decade, botanical insecticides keep attracting more attention from the environment and used worldwide as they are considered as a suitable alternative for synthetic insecticides (Asiry, 2015). Naturally, plants possess several bioactive substances as secondary metabolic constituents, including alkaloids, cucurbitacin, flavonoids, glycosides, terpenoids and other compounds that play central role in controlling mosquitoes (Ghosh *et al.*, 2012; Jan *et al.*, 2021). Several studies have reported mosquito larvicides from many natural plant extracts, such as neem [*Azadirachta indica*] (Howard *et al.*, 2011; Chandramohan *et al.*, 2016), *Artemisia annua* (Masotti *et al*., 2012), *Euphorbia hirta* (Panneerselvam *et al.*, 2013), *Acacia nilotica* (Vivekanandhan *et al.*, 2018) and *Citrullus colocynthis* (Rasool *et al.*, 2020), and other plants. Otherwise, the effectiveness of botanical compounds that act as insecticides against mosquito larvae depends on the plant varieties, parts of the plant used, age of plant parts (immature, mature or senescent), and solvents utilized throughout the extraction (Ghosh *et al.*, 2012).

In the Arabian Peninsula, Arfaj (*Rhanterium epapposum*) is an indigenous and persistent overshadow-shrub traditionally used for medicinal purposes. It is used to manage gastrointestinal disorders and skin infections. This plant usually grows naturally without being cultivated and it is highly adapted to the arid land environment and extensively circulated in deserts, villages, havens, foothills, gorges and lowlands (Petrie, 2007; Kala *et al.*, 2009). The plant has a wide distribution and is found in North Africa and the deserts of Saudi Arabia, Kuwait and the United Arab Emirates (Hellyer and Aspinall, 2005; Petrie, 2007; Omar and Bhat, 2008). More than 107 volatile elements, predominantly terpenoids, have been detected in the essential oil of *R. epapposum* (Yaghmai and Kolbadipour, 1987). In addition, bioactive compounds such as alkaloids, flavonoids, triterpenes, cumarins and tannins have been reported in the ethanolic extracts of aerial parts of *R. epapposum* (Eltahir and Dahab, 2019). However, its use as a mosquito larvicide is not well known. Consequently, this study aimed to assess the larvicidal activities of 3 different extracts of *Rhanterium epapposum* Oliv. leaves against 4th larval instar larvae of dengue fever mosquito vector, *Aedes egypti*, under laboratory conditions.

MATERIALS AND METHODS

Preparation of plant extracts

Aerial parts of *Rhanterium epapposum* were obtained from Al-Nafud desert, north of Hail region, in the northern central part of Saudi Arabia in 2018. These parts were carefully washed, and all pooled crusts such as wildflowers, dust atoms and other unnecessary substances eliminated. The leaves were dried under dark conditions at room temperature (27±2°C) for 4 weeks. The dried leaves were ground using an electric blender, and 50 g milled leaves standardized with 100 mL of three pure solvents: ethanol, acetone (Sigma Aldrich, USA) and distilled water (ELGA Water Purification System, UK). These preparations were left in shaker for 24 h, at room temperature and then centrifuged at 4000 rpm for 20 min (Hettich Zentrifugen Centrifuge Universal 32R Max 4000 RPM

D-78532, Germany). The prepared solution, consisting of the target extract, was transferred to a 250 mL glass beaker. The solvent within the extract evaporated when heated to 60°C, and the rigid material was weighed and dissolved in distilled water. From the stock solution, the dilutions of 5, 50, 500 and 5000 ppm were prepared (Elimam *et al.,* 2009).

Vector rearing

Eggs of dengue fever vector *A. aegypti* were collected from the Laboratory of Public Health Pests, Jeddah Governorate (Al Amana), Saudi Arabia. Enamel and plastic trays, filled with tap water, were used for rearing the new larvae of *A. aegypti*. The reared larvae were fed on aquarium fish foods containing overall nutrients and essential vitamins. Small bowls containing clean water were used for transferring pupae, which were kept in a 50 × 50 × 50 cm cage wrapped in fine mesh net awaiting emergence of adults. Sugar solution (5%, w/v), soaked in cotton wicks dipped in a small conical bottle placed inside the cage, was made available for feeding by male adults. One week-old chicks were used for blood feeding adult females.

Bioassay and mosquito mortality

During the study, WHO standard procedure was followed to determine the larvicidal actions of leaf extracts of *R. epapposum* (WHO, 2005; Ichimori *et al*., 2014). Twenty-five 4^{th} instar larvae of *A. aegypti* were transferred using a dropper to each of the test cups (250 mL), each containing 100 mL tap water, to which five known concentrations from the stock solution (5 g 100 mL^{-1} = 50000 ppm) was used to prepare 5, 50, 500 and 5000 ppm. Five concentrations with four replicates of each concentration across each solvent extract of *R. epapposum* were set up, resulting in a total of 60 tested cups (three extracts × five concentrations × four replicates) being investigated in this study. A control treatment (tap water only) was also included. Mortality was assessed after 24 and 48 h post treatment by checking on the movements of larvae after gentle prodding. Percentage of *A. aegypti* larval mortality was calculated using the following equation:

$$\text{\% of larvae mortality} = \frac{\text{Mean larvae mortality}}{\text{Total of introduced larvae}} \times 100$$

Statistical analyses

Percentages of larval mortality of *A. aegypti* were subjected to the generalized linear model analysis using SAS version 9.2 (SAS, 2009). This model was inclusive for the following factors: the concentrations of solvents (16 levels), observation time (2 levels) and their interaction as a fixed effect. At the same time, the block was included in this model as a covariate random effect. The least significance difference (LSD) was applied to identify differences in treatment means where significant differences were detected. Additionally, the percent larval mortalities were calculated and plotted with consistent concentrations on logarithmic probability paper to complete the corresponding log-concentration Probit lines. The regression lines were produced to determine the lethal concentrations of 50% on *A. aegypti* larvae, and the Probit and toxicity index (LC_{50}) were computed according to Finney (1971) and Busvine (1971).

RESULTS AND DISCUSSION

Mortality of *A. aegypti* due to the type of solvent (ethanol, acetone and water), used in the extraction of *R. epapposum* leaves, against *A. aegypti* varied significantly. The mortality was significantly affected with prolonged exposure time of these extracts (Table 1). The percent larval mortalities were likewise significantly influenced by the interaction between tested extracts and exposure time. At the same time there was no effect on the arrangement of experimental cups on the larval mortality of *A. aegypti* since the experiment was conducted in a homogenous environment.

The ethanolic extract of *R. epapposum* was the best extract that caused higher larval mortality

Table 1: The generalized linear model showing the analysis of variance for the overall effects of solvent concentrations (C), observed time (T), their interaction (C x T) and blocking on the larval mortality of *A. aegypti*

Factors	DF	Sum of squares	Mean square	F value	Pr > $F_{0.5}$
Concentrations of solvents (C)	15	74892	4993	243.9	0.000
Time of exposure (T)	1	1741	1741	85.04	0.000
C × T	15	904	60.23	2.94	0.001
Block	3	48.5	16.17	0.79	0.503
Error	93	1904	20.47		
Total	127	79484			

of *A. aegypti* in terms of concentration used and exposure time as compared to the acetone and water extracts. In contrast, aqueous extract had the lowest effect on larval mortality of *A. aegypti* (Fig. 1**).** At the lowest concentration of 5 ppm, the larval mortalities were 31, 29 and 22% for ethanol, acetone and water extracts, respectively, after 24 h exposure (Fig. 1A). During this exposure time, the ethanolic extract resulted in 98% larval mortality at the higher concentration (50000 ppm). In comparison, the acetone and aqueous extracts showed 72 and 30% larval mortalities, respectively, at the same concentration and exposure time (24 h) (Fig. 1A). After 48 h exposure at 5 ppm, the larval mortality increased due to the effect of *R. epapposum* leaf extracts was more efficient. This increment was in the order of 46, 38 and 25% mortalities for ethanol, acetone and water extracts, respectively (Fig. 1B). Similarly, at the same exposure time (48 h), at the highest concentration of 50000 ppm, the larval mortality increased by 99, 86 and 35% for the ethanolic, acetonic and aqueous extracts of *R. epapposum*, respectively (Fig. 1B).

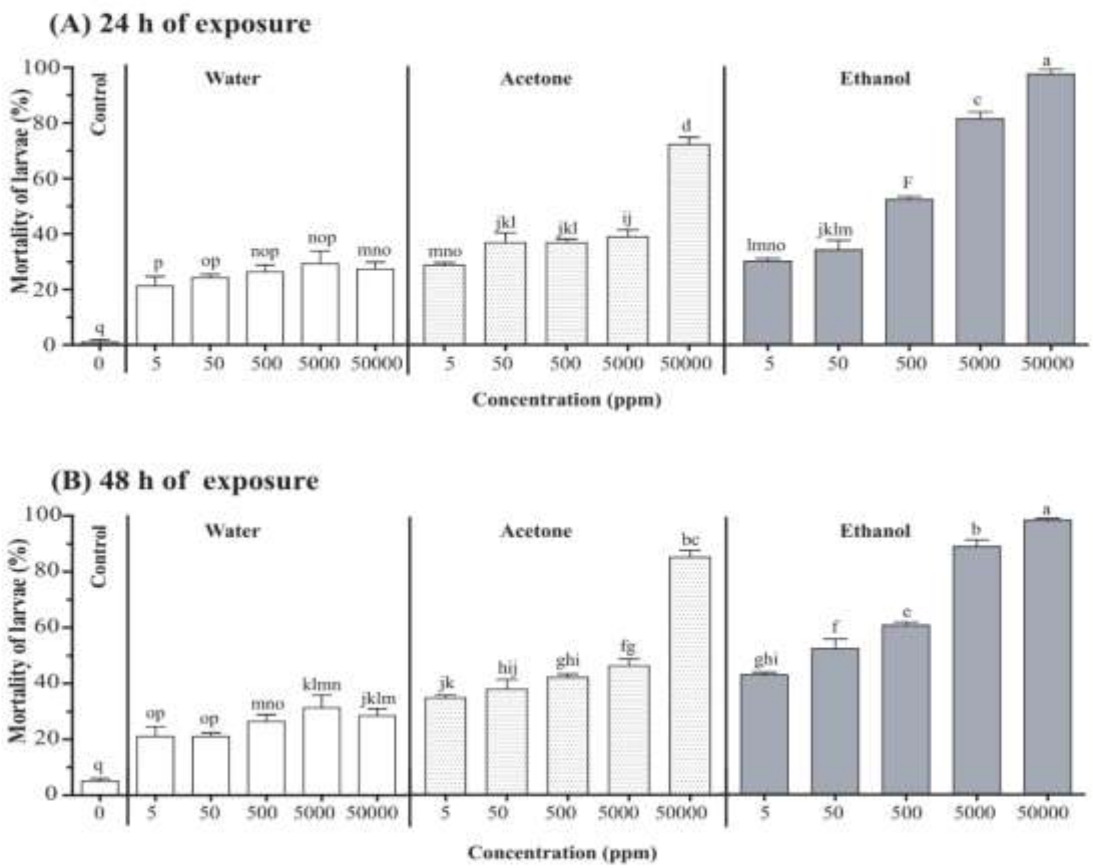

Fig. 1: Larval mortality (%) of *A. aegypti* as affected by different concentrations of solvents of leaf extracts of *R. epapposum* after (A) 24 h and (B) 48 h. Means in each bar followed by the same letter are not significantly different, according to the LSD test

Table 2: Toxicity of acetonic, ethanolic and water leaf extracts of *R. epapposum* against 4th instar larvae of dengue fever mosquito vector after 24 h exposure

Solvents	LC_{50} (ppm)*	95% FL	Slope ± SE	Toxicity index	χ^2 value	DF	Sig.
Ethanol	168.15	18.35-883.81	0.46 ± 0.04	100.00	7.68	3	0.053
Acetone	847.75	Nd	0.27 ± 0.02	19.83	13.06	3	0.004
Water	2278.22	Nd	0.47 ± 0.03	7.38	84.26	3	0.000

*The concentrations are expressed in μLmL^{-1}; FL= fiducial limits, Toxicity index = [(LC_{50} of the most toxic tested compound/LC_{50} of the tested compound)100]. nd = non-detectable.

Table 3: Toxicity of acetonic, ethanolic and water leaf extracts of *R. epapposum* against 4th instar larvae of dengue fever mosquito vector after 48 h exposure

Solvents	LC_{50} (ppm)*	95% FL	Slope ± SE	Toxicity index	χ^2 value	DF	Sig.
Ethanol	27.39	0.12-201.71	0.49 ± 0.05	100.00	13.12	3	0.003
Acetone	568.43	Nd	0.29 ± 0.04	4.82	22.09	3	0.004
Water	2233.25	Nd	0.38 ± 0.03	1.22	33.31	3	0.000

*The concentrations are expressed in μLml^{-1}; FL = Fiducial limits, Toxicity index = [(LC_{50} of the most toxic tested compound/LC_{50} of the tested compound) 100]. nd = non-detectable

The toxicity of ethanolic extract of *R. epapposum* leaves against the larvae of *A. aegypti* was higher, demonstrating a lower LC_{50} value (168.15 ppm) after 24 h. This plant extract displayed more efficacy than acetonic (847.75 ppm) and aqueous (2278.22 ppm) extracts (Table 2). Similarly, the toxicity effect of ethanolic extract increased after 48 h showing the lowest LC_{50} value (27.39 ppm) as compared to the acetone (568.43 ppm) and aqueous (2233.25 ppm) extracts (Table 3). The slope rate of toxicity lines of ethanolic, acetonic and aqueous leaf extracts were 0.46, 0.27 and 0.47; 0.49, 0.29 and 0.38 for 24 and 48 h, respectively (Tables 2 & 3).

The use of synthetic insecticides to control disease vectors (mosquitoes in particular) may sometimes be ineffective due to the resistance developed in vectors to these pesticides and their harmful effects on human health and the environment. Therefore, scientists have sought a safe and sustainable control method such as botanical insecticides that can naturally grow and be collected from different areas worldwide (Khater *et al*., 2019). In this study, the larvicidal efficiency of three solvent extracts from the leaves of *R. epapposum* showed solvent-concentration and time-dependent actions. The ethanolic extract showed higher toxicity and potentially resulted in 98% larval mortality after 24 h exposure at a high concentration of 50000 ppm. This effect, however, was moderate and weak when the larvae of dengue fever mosquito vectors were treated with acetone or aqueous extracts. The toxicity effect increased by increasing the exposure time of treatment extracts of *R. epapposum* leaves at low concentrations (≤5000 ppm). Although studies on testing larvicidal efficiency of *R. epapposum* leaf extracts against mosquitoes are rare, the results of this study are consistent with a comparative study conducted by Asiry *et al.* (2017), who found that the ethanolic extracts from both *R. epapposum* and sweet wormwood, *Artemisia annua* leaves as best extracts for controlling 4th instar larvae of dengue fever mosquito vectors as compared to bitter apple, *Citrullus colocynthis*, and Fattaka, *Pergularia tomentosa* leaves. Kala *et al.* (2009) reported that the evaporative oil structure of aerial parts of *R. adpressum* is a rich source of sesquiterpenoid combinations such as bicyclo [4.4.0] dec-1-ene, 2-isopropyl-5methyl-9-methylene, β-cadinol, α-cadinol, α-eudesmol, β-eudesmol, myristicin, spathulenol and other vital compounds. Awad and Abdelwahab (2016) reported that limonene, sabinene, a-pinene and b-myrcene were dominant compounds in the oil extracted from *R. epapposum* leaves. In addition, Demirci *et al.* (2017) identified 45 constituents from the essential oil extracted from the aerial parts of *R. epapposum* with higher abundances of camphene, myrcene, limonene and a-pinene. Eltahir and Dahab (2019) reported that the ethanolic extract of *R. epapposum* had more abundant alkaloid and flavonoid compounds, and had larvicidal properties against Khapra beetle (*Trogoderma granarium*). Recently, Rajendrasozhan *et al.* (2021) reported several compounds in three extracts (methanol, 80% methanol and aqueous extracts) from the aerial parts of *R. epapposum* such as 2-methoxy-4-vinylphenol in all three extracts. Whereas ethanol, 2-methoxy-acetate in methanol, n-hexadecanoic acid in 80% methanol and 2,3-butanediol in aqueous extract existed in greater volume. Hence, it may be assumed that the chemical configuration of vital oils such as alkaloids, flavonoids, triterpenes, cumarins and tannins in the ethanolic leaf extract of *R. epapposum* could be responsible for its higher toxicity against the larvae of dengue fever mosquito vectors entirely in methanol, 80% methanol and aqueous extracts. Many plant extracts reportedly possess mosquitocidal activities, but very few plant products show the practical utility for mosquito control (Mittal and Subbarao, 2003). Govindarajan and Karuppannan (2011) studied the efficiency of several leaf plant extracts, belonged to the Asteraceae family against some mosquito-borne diseases including dengue fever vector, *A. aegypti.* They showed that the methanol crude leaf extract of *Eclipta alba* caused maximum mortality against the 3rd instar larvae of *A. Aegypti*, followed by chloroform, benzene, ethyl acetate and hexane extracts. Sukhthankar *et al.* (2014) reported that the methanolic leaf extracts of *Chromolaena odorata* caused higher mortality against late instar larvae of disease vectors, *Anopheles stephensi*, *Culex quinquefasciatus* and *A. aegypti* after 24 h of treatment. The aqueous leaf extracts of *Ambrosia arborescens* (Asteraceae) caused a higher toxicity against the 3rd instar larvae of *A. Aegypti* (Bianca *et al.*, 2018).

The present study focused on the possible use of botanical insecticides against dengue fever mosquito vectors, especially essential oils that are considered environmentally safe alternatives to synthetic insecticides. These botanical insecticides presumably have broad-spectrum effectiveness, and prorated trait in their mode of action. These botanical insecticides contain adequate chemical constituents that could function as repellents, contact insecticides, fumigants, anti-feedings, or could disrupt and obstruct the energetic functions of disease-vectors (Shaalan *et al.*, 2005; Tang *et al*., 2007; Rozman *et al.*, 2007; Ghosh *et al.*, 2012). In west of Saudi Arabia, dengue fever vector, *A. aegypti*, is considered an important mosquito species responsible for dengue fever spread (Alshehri, 2013). In past few years, the number of dengue fever cases has multiplied in Jeddah province (Al-Ghamdi *et al.*, 2009) which has led to the extensive use of insecticides to control the vector. Insecticides have adverse side effects on human health and environment (Winqvist *et al.*, 2011). This study has identified an alternative method to synthesized insecticides for controlling the 4th instar larvae of dengue fever vector, *A. aegypti*, by observing larvicidal efficiencies of the three extracts from *R. epapposum* leaves. In conclusion, more studies on chemical constituents, larvicidal effectiveness and bioactive chemical ingredients constancy of ethanolic extracts of *R. epapposum* leaves need to be conducted to have a sustainable control method for such disease-causing mosquito vectors.

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